

REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claim Amendments

Previous claims 2, 25, 27, 30 and 31 have been canceled.

Previous claims 1, 3, 24, 26 and 29 have been re-written as new claims 32, 33, 34, 35 and 36 respectively.

Claims 4, 5, 6, 8, 9, 10, 12, 13, 14, 15, 18 and 21 have been amended to reflect the changes in new claims 32 and 33.

Claims 7, 11, 16, 17, 19, 20, 22 and 23 remain unchanged.

Claim 28 stands withdrawn.

Applicants hereby reserve the right to file a continuation or divisional application on the subject claimed prior to this amendment. Applicants submit that no new matter has been added by these amendments and hereby request their entry. Claim 28 is withdrawn and Claims 4-23, and 32-36 are now pending in the application.

Information Disclosure Statement

Applicants are resubmitting an Information Disclosure Statement herewith that includes a copy of WO 2003/050544. Applicants request that the Examiner consider WO 2003/050544.

Claim Rejections under 35 U.S.C. § 101

Claims 27 and 30-31 were rejected under 35 U.S.C. §101 because the claimed recitation of use allegedly results in an improper definition of a process, i.e. results in a claim which is not

a proper process claim under 35 U.S.C. 101. Applicants have canceled these claims rendering this rejection moot.

Claim Rejections under 35 U.S.C. §112, first paragraph

Claims 1-27 and 29 were rejected for allegedly failing to comply with the written description requirement. The Examiner states that the subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

In an effort to expedite examination, Applicants have deleted the following terms:

(i) “affinity group”, “use of an affinity group”, and “without use of an affinity group” in claims 1, 3, 24, 26 and 29 (new claims 32 -36);

(ii) “molecules are derivatized prior to analysis” in claims 1, 3 and 29 (new claims 32, 33 and 36).

(iii) “derivatives” in claim 29 (new claim 36).

Applicants request withdrawal of this rejection.

Claim Rejections under 35 U.S.C. §112, second paragraph

Claims 1-27 and 29 were rejected as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The rejections under 35 U.S.C. §112, second paragraph, will be addressed below.

(i) Applicants have deleted the terms “the molecules are derivatized” and “derivatized prior to analysis” from claims 1, 3, 26 and 29 (new claims 32, 33, 35 and 36).

(ii) Applicants have deleted the terms, “reagents are labeled”, “affinity group” and “use of an affinity group” from claims 1, 3, 24, 26 and 29 (new claims 32-36).

(iii) Applicants have provided proper antecedent basis for “the reagents” and “the molecules” in claims 1, 3, 24, 26 and 29 (new claims 32-36).

(iv) Applicants have deleted the term “with at least two differential isotope labeled reagents” from claim 1 (new claim 32).

(vi) Applicants have clarified the number of chemically distinct reagents and isotopically distinct combinations in claims 1, 3, 24, 26, and 29 (new claims 32-36).

(vii) Applicants have amended claim 1 and 3 (new claims 32 and 33) by deleting the term “wherein the differential isotope labeled reagents results in differential isotope labeled derivatives” and “wherein the differential isotope labeled reagents result in a reductive alkylation” respectively.

(viii) Applicants have amended claim 3 (new claim 33) by deleting the terms “with isotope labeled reagents” and “with differential isotope labeled reagents” respectively.

(ix) Applicants have amended claim 5 by deleting the term “the step of reacting the molecules with differential isotope labeled reagents” and inserted the term “step(ii)”.

(x) Applicants have deleted the term “the differential isotope labeled” from claim 15.

(xi) Applicants have deleted the term “with the molecules” from claim 24.

(xii) Applicants have placed the term “for quantitative analysis by mass spectrometry” after the term “A preparation” in claim 26 (new claim 35).

(xiii) Applicants have provided proper antecedent basis for the term “the molecules” in claims 26 and 29 (new claims 35 and 36).

(xiv) Claim 26 has been re-written as new claim 35.

(xv) Claims 27, 30 and 31 have been deleted.

(xvi) Claim 29 has been re-written as new claim 36 to make clear that one reacts the (a) three samples of cellular extracts with (b) the three combinations of differential isotope labeled reagents.

In light of the amendments and the arguments presented, Applicants submit that the rejections under 35 U.S.C. §112 has been obviated and therefore request that the rejections be withdrawn.

Claim Rejections under 35 U.S.C. § 102

The Examiner has rejected claims 2-6, 8-15, 17-23, 25 and 29 as allegedly anticipated by Aebersold et al. (US 6,670,194) (hereinafter “Aebersold et al.”).

In the response to arguments, the Examiner stated that the limitation “labeled without use of an affinity group” is not sufficient because the affinity group does not intervene between the linker and the protein reactive centre. Applicants have deleted this term from the claims.

The Examiner further stated that the “position that the present invention ‘involves placement of deuterium and/or carbon-13 in methyl amine ($N-(CH_3)_2$ or $N-(CD_3)_2$ ’ is not persuasive because Applicants appear to rely upon limitations that do not appear in the rejected claims.” Applicants have amended claims 3, 26 and 29 (new claims 33, 35 and 36) by (i) inserting the term, “at an alkylamine”, (ii) by inserting the term “providing at least three combinations of differential isotope labeled reagents...”, and (iii) by inserting “at least three samples...”. Support for these amendments can be found throughout the specification, and in particular in Figures 10, 11 and 12, paragraph 0074-0076, 0078, 0079, 0088, 0089, 0092, 0093, 0097 (in particular Table 1), and Examples 1, 4, 7, 8 and 9.

As discussed in the last Office Action response, Aebersold et al. disclose a method to analyze proteins using a composite molecule of the formula A-L-PRG, where A is an affinity label (also known as an affinity group) that selectively binds to a capture reagent, L is a linker group which is differentially labeled with one or more stable isotopes and PRG is a ‘protein

reactive group' that selectively reacts with a protein functional group or is a substrate for an enzyme.

Applicants will contrast the method of Aebersold et al. versus that of the present invention:

1. Aebersold et al. use a linker whereas the present invention does not.

The linker is described in column 4 lines 1 to 12, column 9 lines 50 to 67 and column 10 lines 1 to 29. It is a separate entity from the protein reactive group (PRG) as described on column 4 lines 6. "In general, the affinity labeled protein reactive reagents of this invention have three portions: an affinity label (A), covalently linked to a protein reactive group (PRG) through a linker group (L)



Examples of linkers are shown in columns 51 to 74. A preferred linker has the formula:



The function of the linker is twofold: (i) to attach the affinity group to the protein reactive group and (ii) to provide the differential isotopic label. It does not react with the protein to be analyzed.

2. Aebersold et al. describe a linker that can contain stable isotopes whereas the present invention describes multiple sets (combinations) of derivatization reagents each of which contain, for example an aldehyde and reducing agent, both of which can contain stable isotopes to provide differential derivatization.

In contrast to the present invention (in which no linker is used), Aebersold et al. teach that the linker is labeled, not the protein reactive group. Column 4 lines 8 to 11 state: "The linker may be differentially isotopically labeled, e.g. by substitution of one or more atoms in the linker with a stable isotope thereof. For example, hydrogens can be substituted with deuteriums

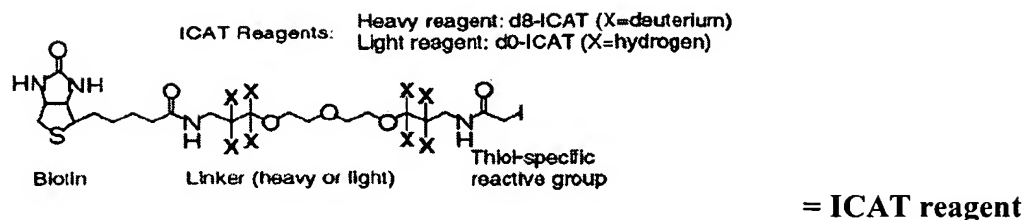
or C¹² with C¹³”. In contrast, in the present invention the set or combination of reagents are isotopically labeled and function in an analogous manner as the PRG of Aebersold et al. For example, in the case of an aldehyde and a reducing agent, the aldehyde functions in an analogous manner as the PRG of Aebersold et al., and reacts directly with the molecule to be derivatized (the amine with an active hydrogen) and the reducing agent then reacts with the product of the first reaction between the aldehyde-amine intermediate product to form a second and final product, the alkylamine.

3. Aebersold et al. teach that the linker (which is isotopically labeled) should not react with the components in the sample i.e. the molecules to be analyzed.

Column 9, lines 50 to 53 state: “The linker group (L) should be soluble in the sample liquid to be analyzed and it should be stable with respect to chemical reaction e.g. *substantially chemically inert with components of the sample as well as A and CR groups*”.

In contrast, the present invention teaches that the entity that is isotopically labeled must react with the components in the sample in order to produce isotopically differentiated derivatives at the alkylamine. Aebersold et al. do not teach any instance of a PRG that contains differential isotopes.

Applicants provide an example, which will exemplify the differences between the teachings of Aebersold et al. and the present invention. Aebersold et al. describe the use of an aldehyde in the context of a protein reactive group (PRG) (col. 10, lines 50-52) which would then be coupled to an isotope labeled linker (L) (col. 9, lines 60-65) and an affinity group. The composite molecule and the reaction with a peptide NH₂-EACDPLR-COOH would resemble the following:



The ICAT reagent reacts with thiol groups on cysteine residues (represented by "C" in the model peptide $\text{NH}_2\text{-EACDPLR-COOH}$). No alkylamine derivative is formed with the reagents described by Aebersold which, due to their size, do not react quantitatively with amines..

In contrast, the present invention requires a *set or combination of at least two reagents* that are isotopically labeled and react with the amine group of the peptide (represented "NH₂"). A reaction with the sample peptide $\text{NH}_2\text{-EACDPLR-COOH}$ would proceed as follows:



Table 1 of the specification (found on page 19, paragraph [0097]) lists examples of the at least three combinations of differential isotope labeled reagents, wherein each combination comprises least two chemically distinct reagents and each combination is isotopically distinct and is reproduced for the Examiner's convenience below. Note that there are no linkers or affinity groups. The reagents themselves contain differential isotopes.

Table 1 Differentially labeled formaldehyde and sodium cyanoborohydride

Formaldehyde	Reducing Agent	Solvent	Added Mass per NH ₂	Δ Mass per NH ₂
CH ₂ O	NaCNBH ₃	H ₂ O	28.0316	0.0000
CH ₂ O	NaCNBD ₃	D ₂ O	30.0474	2.0158

$^{13}\text{CH}_2\text{O}$	NaCNBH_3	H_2O	30.0316	2.0000
$^{13}\text{CH}_2\text{O}$	NaCNBD_3	D_2O	32.0474	4.0158
CD_2O	NaCNBH_3	H_2O	32.0632	4.0316
CD_2O	NaCNBD_3	D_2O	34.0790	6.0474
$^{13}\text{CD}_2\text{O}$	NaCNBH_3	H_2O	34.0632	6.0316
$^{13}\text{CD}_2\text{O}$	NaCNBD_3	D_2O	36.0790	8.0474

For example, Table 1 lists formaldehyde as a reagent. The formaldehyde can be CH_2O , $^{13}\text{CH}_2\text{O}$, CD_2O , $^{13}\text{CD}_2\text{O}$ (i.e. 4 different isotopic forms of formaldehyde). There are at least two chemically distinct reagents for each combination, and each combination is isotopically distinct from the others. The isotopic reagents used in the present invention greatly simplifies the chemistry involved while, at the same time, achieving the goal of attaching different isotopic labels to different protein samples. The practical advantage of simple chemistry is that the differential reagents described here do not interfere with subsequent mass spectrometric analysis as a result of their small size. Indeed the technique taught by Aebersold et al. and sold by Applied Biosystems as "ICAT" reagents has been replaced by a "cleavable ICAT" reagent (a copy of which was enclosed for the Examiner's convenience in the last response) that involves removal of the affinity group after affinity purification to reduce interference with mass spectrometric detection. Additionally, as discussed above Aebersold et al. use a linker group containing isotopes, specifically deuterium atoms, attached to aliphatic carbons to produce differential reagents (i.e. $-\text{CH}_2-$ or $-\text{CD}_2-$), such as in Aebersold et al. schemes 1, 2, 19 & 21. In contrast, the present invention involves placement of deuterium and/or carbon-13 in methyl amine ($\text{N}-(\text{CH}_3)_2$ or $\text{N}-(\text{CD}_3)_2$). The advantage of placement of the isotopes at this position is that the derivatised molecules co-elute under reverse phase chromatography (see Figure 6B in the current application) whereas the reagent described by Aebersold et al. do not co-elute. Indeed, in the "cleavable ICAT" reagent mentioned above, carbon-13 is used in place of deuterium to improve chromatographic co-elution. The claims have been amended accordingly.

In addition, as discussed in [0074], the present invention can produce up to eight isotopically unique reagents by the use of formaldehyde, deuterated formaldehyde, carbon-13 formaldehyde, carbon-13/deuterated formaldehyde, sodium cyanoborohydride and sodium

cyanoborodeuteride, in a simple, two-step reaction. The use of a two step reaction provides two opportunities to introduce isotopes, whereas, the one-step reaction described by Aebersold et al. do not have such flexibility. Therefore, the present invention could be used to analyze up to eight samples simultaneously. Although Aebersold et al. state in claim 1 that the technology can be used for two or more samples, Aebersold et al. do not provide sufficient information or any examples of reagents that are capable of analyzing more than two samples.

Given that Aebersold and co-authors describe reagents with amines bearing active hydrogens such as primary amines (Aebersold scheme 3 contains a $-NH_2$ group) and also methyl amines (Aebersold scheme 13 contains a $-N-(CH_3)$ group) but do not describe isotopic derivatization of these amines and the resulting increased performance, demonstrates that the technology described here was not anticipated by Aebersold et al. nor would it be obvious to those skilled in the art.

Further, as discussed above the present invention does not employ a separate affinity group (A). The use of a separate affinity tag and a linker is a central component of the technology developed by Aebersold et al. They elaborate on the beneficial effects of selective enrichment (col. 14, lines 47-50). Accordingly, Applicants submit that the system disclosed in Aebersold et al. employs an affinity group and linker group, and does not label the molecules of interest at the alkyl amine position, therefore cannot anticipate the presently claimed invention.

As such, Applicants respectfully request withdrawal of the objections.

Claim Rejections under 35 U.S.C. 103(a)

Claims 1, 4-15, 17-24 and 27 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Aebersold et al. (US 6,670,194) in view of Figeys et al. (US 2002/0076817) (hereinafter "Figeys et al.").

Claim 16 stands rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Aebersold et al. (US 6,670,194) and Figeys et al. (US 2002/0076817) as applied to claims 1 and

15 and further in view of Vandekerckhove & Gevaert (US 6,670,194) (hereinafter “Vanderkerckhove”)

First, to establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, a prior art reference (or references) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants’ disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

A. Aebersold et al. in view of Figeys et al.

Claim 1 (new claim 32) has been amended by adding the step of providing at least three sets of differential isotope labeled reagents, wherein each set of differential isotope labeled reagents comprises at least two reagents. As described above, Aebersold et al. does not teach the method as currently claimed. Further, the teachings of Aebersold et al. do not suggest the currently claimed invention. The citation of Figeys et al. does not cure this defect. As discussed in the last Office Action response, Figeys et al. does not supply the necessary teachings to arrive at the currently claimed invention in providing at least three sets of differential isotope labeled reagents, in which each set comprises at least two chemically distinct reagents.

Accordingly, the claims are not obvious in view of Aebersold et al. and Figeys et al.

B. Aebersold et al. and Figeys et al. further in view of Vanderkerckhove

The Examiner has rejected claim 16 on the basis of obviousness with regard to Aebersold et al. (US 6,670,194) in view of Figeys (US 2002/0076817) and Vandekerckhove and Gevaert (US 2004/0005633).

For the same reasons as described above, the combination of Aebersold et al. and Figeys et al. does not teach the methods of the amended claims. Vandekerckhove teaches a multi-step procedure that requires the peptides be separated into fractions via chromatography prior to any chemical alteration. (col. 1, paragraph [003]). Vandekerckhove does not teach analysis of “at least three samples of differential isotope labeled derivatives of molecules”. Vandekerckhove teaches the analysis of “altered and non-altered” peptides (see paragraph [0041]) also described as “flagged versus “unaltered peptides” (see paragraph [0050]). Therefore, Vandekerckhove does not provide “at least three combinations of differential isotope labeled reagents”. Accordingly, the combination of Aebersold et al., Figeys et al. and Vandekerckhove does not teach the method of dependent claim 16.

In light of the arguments presented above, Applicants respectfully request that both rejections under 35 U.S.C. §103 be withdrawn.

Conclusion

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even

entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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